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Changes in serum triiodothyronine kinetics and hepatic type I 5′-deiodinase activity of cold-exposed swine

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Department of Medicine, Endocrine Service, Madigan Army Medical Center, Tacoma, Washington 98431–5000; Thermal Stress Adaptation Program, Naval Medical Research Institute, Bethesda, Maryland 20889–5055; Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814–4799; Veterans Affairs Medical Center, Palo Alto, California 94304; and Department of Animal Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

Reed, H. Lester, Marilee Quesada, Robert L. Hesslink, Jr., Michele M. D'Alesandro, Marguerite T. Hays, Robert J. Christopherson, Brian V. Turner, and Bruce A. Young. Changes in serum triiodothyronine kinetics and hepatic type I 5′-deiodinase activity of cold-exposed swine. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29): E786–E795, 1994.— Swine exposed to cold air have elevated serum values of total triiodothyronine (TT_3) and free T_3 (FT_3). To characterize the mechanism of these increases, we measured in vivo kinetic parameters after a bolus intravenous injection of ^{125}I-labeled T_3 by use of both multicompartmental (MC) and noncompartmental (NC) methods and in vitro hepatic type I iodothyronine 5′-deiodinase (5′D-I) activity. Ten ad libitum-fed 5-mo-old boars were divided into two groups, living for 25 days in either control (22°C) or cold (4°C) conditions. Cold-exposed animals consumed 50% more calories than control animals but showed no difference in total body weight, percent body fat, or plasma volume. Thyroid gland weight was increased 86% ($P < 0.004$), as was serum total thyroxine (TT_4) (48%), free T_4 (FT_4) (61%), TT_3 (103%), and FT_3 (107%), whereas serum thyrotropin (TSH) was not different in cold-exposed compared with control animals. The T_3 plasma clearance rate was similar between groups when both MC and NC techniques were used. However, T_3 plasma appearance rate (PAR) was elevated in cold-treated animals 110% over controls by MC ($P < 0.001$) and 83% by NC methods ($P < 0.001$). The animal total hormone pool of T_3 was increased 76% (MC) and 53% (NC) compared with control ($P < 0.01$). The Michaelis constant of hepatic 5′D-I was not different between groups, but the maximum enzyme velocity increased (106%; $P < 0.02$). Therefore cold exposure for 25 days is associated with increased energy intake, thyroid size, T_3 PAR, and hepatic 5′D-I activity with little change in serum TSH.

adaptation; noncompartmental methods

CONSIDERABLE INFORMATION EXISTS regarding the role of triiodothyronine (T_3) in brown adipose tissue- (BAT) mediated thermogenesis (25). This specialized adipose tissue contributes significantly to circulating T_3 in cold-exposed rodents (25). BAT is found in human infants and patients with hibernoma and pheochromocytoma (2). Its role, however, in both adult human thermoregulation (1) and cold-associated increases in T_3 production (23) remains uncertain. Mature swine also lack histological BAT. When this species is exposed to cold, the animals do not possess BAT uncoupling protein as do rodents (29). Thus the influence of cold exposure on the metabolism of T_3 in swine, which lack BAT as a possible source of the hormone, should provide information more applicable than rodent data as a model for human physiology and thermoregulation (7).

The outer ring 5′ iodine of thyroxine (T_4) is removed to form T_3 in peripheral tissues by the enzyme 5′-deiodinase (5′D). Several forms of this enzyme exist, including type I (5′D-I), found primarily in liver and kidney, and type II (5′D-II), located in brain, pituitary, and BAT (14). Six- to eightfold increases in 5′D-II activity of BAT from cold-exposed rodents contribute significantly to increased circulating values of T_3 in this species. By contrast, the 5′D-I activity that supplies circulating T_3 under normal conditions in the rat is not consistently increased with cold exposure (20, 21, 25). We therefore hypothesized that if the T_3 production rate is increased with cold in a species lacking BAT, an alternate hormone source should be present to offset the missing BAT contributions.

Swine are known to increase energy intake, serum concentrations of T_4 and T_3, and the disposal of T_4 with constant cold exposure (12, 13, 16). We studied the mechanism of increases in serum T_3 by comparing both the in vivo ^{125}I-labeled T_3 degradation and in vitro generation of T_3 in animals exposed to either 4 or 22°C for 25 days. We hypothesized that an increased serum T_3 concentration would be associated with either a decreased plasma clearance rate (PCR) and unchanged production rate (PR) or with unchanged PCR and increased PR. Because the T_4 disposal rate (DR) is increased in this swine model, we favored an augmented T_3 production to account for the change. Furthermore, if hepatic 5′D-I activity were contributing to the elevated serum T_3 concentrations, the activity of this enzyme should either increase or be unchanged in the presence of increased serum T_4.

METHODS

Animal characteristics and environmental exposure protocol. Protocols using 10 mature 5-mo-old boars (*Sus scrofa*) of the Large White breed (Pig Improvement, Calgary, AB, Canada), were approved for use by the Committee for the Use and Care of Animals at the Naval Medical Research Institute, Bethesda, MD, and the University of Alberta, Edmonton, AB, Canada. All studies were carried out in Edmonton from February to March, 1990.

For ~4 mo preceding the study, all animals were housed in climate-controlled conditions (20 to 25°C) and fed a highly digestible energy feed ad libitum [160 mg protein and 3.2 kcal

digestible energy per g dry matter (DM)]. One day before temperature treatments, the animals were housed in metabolic crates within the climate chambers set to thermoneutral conditions (22°C) for familiarization. The floors of the metabolic crates were constructed of stainless steel rods 12.7 cm apart with reinforced clear Plexiglas inserts for walls and an open top. The floor of the crate was 65 cm from the floor of the climate chamber, and air freely circulated through the crate without significant lateral drafts.

Animals were randomly assigned to the control or cold condition. The temperature in the cold chamber was gradually lowered over 5 days to the predetermined value of 4°C, with a range of 1.8–7.3°C; the control chamber remained at 22°C, with a range of 20.4–22.8°C. The chambers had floor areas of 40 and 50 m^2, respectively, and had similar airflow (< 0.5 m/s) and relative humidity (45–60%). Cyclic lighting was maintained with automatic timers set to illuminate between 0900 and 2100 h. The animals received water by an automatic nipple fountain and feed through a front end trough ad libitum. The feed contained 3.2 kcal/g, 16% crude protein, and 92% DM composed of 51% barley, 30% wheat, and 13% soybean meal. The animals were weighed weekly in a rolling metabolic cage similar to their chamber crates, a procedure allowing no more than 10 min/wk outside of the treatment environment. The animal groups were matched for weight and growth rate by allowing energy intake to increase in the cold-exposed group to achieve this similarity. Background music, as is customary, was provided to aid in normalization of environments. The animals were housed in the temperature treatment chambers 25.2 ± 2.8 days before the kinetic study and an additional 4.2 ± 0.4 days until necropsy. There was no group difference in either the duration of temperature exposure or time to death.

One week before the kinetic studies (see below), two Silastic catheters were inserted into the external jugular vein. They were positioned, brought to the surface in the midback region, and used for sampling and injecting radiolabeled hormones. The catheters were maintained patent by flushing with a solution of heparin sodium (100 U/ml) at 12-h intervals. One catheter was positioned distal for injection, and the other one was more proximal. This arrangement minimized contamination of the sampled blood with the injected substances. The catheters were placed while the animals were given a general anesthesia with a 2% halothane-oxygen mixture. Recovery from anesthesia and surgery was verified by normalization of feeding habits and a stable rectal temperature compared with the preoperative value. Prophylaxis against catheter-related infection was carried out in all animals with ampicillin (500 mg iv) every 12 h from the postoperative period until necropsy.

Kinetic experiments. L-3,5,3′-^{125}I-labeled triiodothyronine ([^{125}I]T_3) was purchased from New England Nuclear (Wilmington, DE; sp act 2,200 Ci/mmol). The purity was confirmed by high-pressure liquid chromatography (HPLC), and it was shown that > 98% of ^{125}I was in the form of [^{125}I]T_3 at the time of analysis. The radiopharmaceutical was diluted with sterile water and sterilized by filtration through a 0.2-μm filter (Millipore, Bedford, MA). Thyroidal ^{125}I uptake was blocked by the oral ingestion of a volume of saturated potassium iodide (KI) solution containing 250 mg of KI. This solution was delivered on apple slices given twice a day starting 24 h before the kinetic protocol and continuing through the 1st day of sample collection (250 mg iodide per 5 drops; Roxane Laboratories, Columbus, OH) (16, 24). KI was administered to minimize differences between human and swine kinetic protocols (24). [^{125}I]T_3 was diluted to a volume of 5 ml with 1% autologous animal serum 5 min before injection; it was then administered to nonfasted animals as a bolus intravenous injection of ~36.4 pmol, and the catheter was flushed with an equal volume of the stock heparin solution. Catheter dead space volume was replaced with this heparin solution. Kinetic studies with the control and cold-exposed animals were carried out at the treatment temperatures. The animals were studied in a pattern that alternated tracer injections between control and cold-exposed animals to limit between-group methodology differences. The dose vial, syringe, catheter, and three-way stopcock arrangement contained < 5% residual radioactivity, and the individual dose was corrected for this residue. There was no group difference with respect to absolute administered dose. Six milliliters of venous blood from the proximal port of the catheter were obtained before and at 0.08, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 8.0, 24, and 48 h after injection. A total of 90 ml of whole blood (< 2% of the total blood volume) was collected. Blood samples were allowed to clot at room temperature for a minimum of 2 h. Serum was separated by centrifugation at room temperature (1,000 *g*, 10 min) and stored at −70°C.

Further animal procedures. Approximately 4.2 days after the [^{125}I]T_3 kinetic studies, nonfasted animals were weighed, and blood samples were obtained just before a lethal injection of 0.3 mg/kg T-61 (Hoechst, Regina, SK). Animals were killed in the exposure temperature and were then immediately transported to the necropsy room.

Three hours before death, a second dose of [^{125}I]T_3 was administered to measure the tissue uptake later at death. As with the kinetic studies, the animals received saturated KI solution orally for the 24 h before death. This second intravenous dose of ~36.4 pmol [^{125}I]T_3 (prepared, handled, and administered exactly like the kinetic dose) was delivered as part of a companion study (22). Two animals were killed without receiving the second dose. The residual radioactivity from the kinetic study dose was calculated to comprise < 3.0% of the radioactivity found in the liver 3 h after the second injection of [^{125}I]T_3.

Pericapsular fat and connective tissue were quickly removed from the thyroid gland before weighing. The wet weight of the liver and thyroid gland was then obtained. Tissue samples and homogenates were frozen at −70°C within 20 min of death. Liver homogenates were prepared as described under the methods of the 5′D-I assay.

Hormone extraction procedure. The [^{125}I]T_3 radioactivity in the serum samples and standards was measured as previously described (24). Briefly, an aliquot of serum was acidified to pH 3.0 with trichloroacetic acid (TCA), extracted with 4 volumes of 9:1 (vol/vol) ethyl acetate-butanol, and centrifuged at 800 *g* to promote phase separation. The ^{125}I content of the organic phase was determined in a Gamma Trac 1193 counter (TM Analytic, Elk Grove, IL) with a 75% counting efficiency. Aliquots of [^{125}I]T_3 stock, handled in a manner identical with the serum samples, were used as counting standards as well as controls for determination of the extraction coefficient. The aqueous phase routinely extracts > 99% of the free iodide by this technique (24). HPLC separation of the organic phase confirmed < 2% of the ^{125}I to be free iodide or butanol nonextractable iodine. This method extracts 81.6 ± 1.5% of labeled T_3 into the organic phase. Serum samples obtained 5 min after injection had an extraction coefficient of 81.7 ± 0.8%, which was identical to the standard, and confirmed by HPLC, to be > 98% [^{125}I]T_3.

Kinetic analysis. Kinetics of the serum [^{125}I]T_3 data were analyzed separately by multicompartmental (MC) and noncompartmental (NC) methods, and the analyses were done by different authors. Iterative least-squares curve fitting was used in both modeling methods.

MC analysis was done using the Simulation, Analysis, and Modeling methodology and applying a three-compartment mammillary model that has been widely used for T_3 kinetics in other species. Before analysis, each pig's data were corrected for the measured plasma volume to obtain the percent dose in the plasma pool at each point in time. These values were used in the model as the content of *compartment 1*, the circulating [^{125}I]T_3 compartment.

The model shown in Fig. 3 is not strictly identifiable in the form illustrated when all parameters are allowed to fit, yet the separate kinetics of the slow- and fast-exchanging tissues are of interest. To handle this problem, a sequence of model fits was employed. First, the data for each pig were fitted to two "limiting" models, in which $k_{0,3}$ or $k_{0,2}$ was fixed at zero, so that all exit from the system was from *compartment 2* or *compartment 3*, respectively. These fits provided outer bounds for the kinetic parameters for each pig. Results of this independent analysis of the two limiting model forms (shown in Table 3) showed that $k_{2,1}$ differed by only ~10% between the two extreme solutions. Hence, for the definitive solutions in which metabolism in both exchange compartments was examined, $k_{2,1}$ was fixed at its midpoint in the two limiting solutions. This left only five adjustable parameters, so that the model was now theoretically identifiable. The results of this final set of model solutions were used in the steady-state calculations and in the comparisons with the NC analyses.

The NC kinetic solution for comparison with an earlier human study (24) was based on a two-exponential fit of the serum [^{125}I]T_3 decay data for each pig. The eigen values from these fits were then used to calculate the NC steady-state kinetic parameters (24).

The steady-state kinetic parameters from both the three-compartment and the NC solutions were then multiplied by the individual pig's serum T_3 levels to calculate the T_3 plasma disappearance rates [which equate to the minimum bound for production rate (PR) at steady-state] and the minimum bounds for T_3 content of the various compartments for the entire system (Q). T_3 produced and degraded in peripheral tissues without ever entering the blood will not appear in these steady-state calculations. Thus the PR and Q values calculated in this study represent lower bounds on T_3 production rate and tissue contents. The upper bounds for PR and Q in the three-compartmental analyses presented here averaged 28 and 38% greater in the control and cold-exposed pigs, respectively, than the lower bounds for the three-compartmental solutions presented in RESULTS. This underestimation is even more marked for the NC than for the MC solution method.

All steady-state parameters were normalized for body weight before statistical analysis.

Plasma volume and body composition measurements. Plasma volume and body composition were determined ~4 days before the kinetic studies at 0900–1100 h by injection of Evans blue dye and ^3H_2O, respectively. Plasma samples were collected at 2, 6, and 24 h for calculation of these distribution spaces. The Evans blue dye concentration was determined spectrophotometrically, and the percent injected dose per liter was expressed for each animal. Extrapolation to *time 0* provided estimates of plasma volume. Body composition was determined with live body weights and ^3H_2O distribution spaces (300 μCi/animal; sp act 70.27 μCi/g; New England Nuclear). The ^3H_2O activity was determined in the same Packard Tri-Carb liquid scintillation analyzer, which has a counting efficiency of ~47%.

Body weights were determined 1 day before injection, and hematocrit (Hct) was measured by microcentrifugation the day of injection. Blood volume was calculated by (Evans blue distribution space) × [1.0 − (0.96 × Hct/100)]$^{-1}$.

Miscellaneous assays. Immediately before injection of [^{125}I]T_3 for the kinetic studies, blood samples were obtained for values of serum total thyroxine (TT_4), free T_4 (FT_4), total T_3 (TT_3), free T_3 (FT_3), thyrotropin (TSH), and Hct. Serum TT_4, FT_4, TT_3, and FT_3 determinations were carried out in quadruplicate with commercially available radioimmunoassay (RIA) kits that have been previously used with swine (Diagnostic Products, Los Angeles, CA) (12). Lower limits of detection for these assays were 4.0 nmol/l, 1.0 pmol/l, 0.11 nmol/l, and 0.77 pmol/l, respectively. The coefficient of variation (CV) of these assays, when repeated samples were analyzed on the same day, was <3.8 and <5.4% for samples measured on different days. Serum TSH was assayed using a commercially available kit (TSH-K-PR, CIS-US, Bedford, MA) with specificity for swine and sheep (12). This assay has an intra-assay CV of <5.0%. Hct values were measured by microcentrifugation and hemoglobin values by spectrophotometric analysis.

One gram of tissue from a few of the animals (liver, thyroid, skeletal biceps muscle) was homogenized, as described below, with tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.4) and precipitated (pH <4.0) with TCA. Extraction was carried out with 4 volumes of ethyl acetate-butanol 9:1 (vol/vol). The organic solvent was dried with nitrogen, and the iodothyronines were separated using HPLC with 30-s collections and a flow rate of 1.5–2 ml/min. Ultraviolet spectrophotometry (Waters, Milford, MA) was used for detection. Stock solutions of T_3, T_4, and reverse T_3 (rT_3; Sigma Chemical, St. Louis, MO) were used to identify migration peaks. Extraction efficiency was monitored using incubations of [^{125}I]T_3 and was found to be consistent for the samples studied and similar to that for serum (~80%). The relative amounts of iodothyronine found in a single tissue had a pooled between-animal CV range of 12.7–36.5%.

Tissue homogenization. Liver tissue was dissected into 2-g sections and homogenized in 9 volumes of cold (4°C) Tris buffer (0.2 M, pH 7.4; Sigma Chemical) containing EDTA (2 mmol/l) and dithiothreitol (DTT; 2 mmol/l) (Buffer A) using a Bio-Homogenizer (Biospec Products, Bartlesville, OK). Aliquots of the homogenates were frozen at −70°C within 20 min of death. Tissue samples of liver and thyroid were assayed by drying at 80°C for 24 h to determine percent DM. Kjeldahl digestion was used to estimate crude protein, and lipid content was analyzed after ether extraction.

5'D-I assay. Measurement of 5'D-I kinetic parameters was by modification of the method of Leonard and Rosenberg (17). ^{125}I-labeled T_4 was chosen as the substrate of the assay to compare our results with earlier studies carried out with swine (3, 26, 27). L-thyroxine ([^{125}I]T_4; sp act ~1,700 μCi/nmol, New England Nuclear) was used as the substrate for measuring outer-ring deiodination with commercial purity stated as >95%. This tracer was further purified the day before any enzyme determination by passage through a Sephadex G 50–125 column (Sigma Chemical) equilibrated with Buffer A. By use of this technique, substrate radiopharmaceutical was determined by HPLC to be >97% pure. Unlabeled thyroxine (Sigma Chemical) was dissolved in 0.1 N NaOH and diluted with Buffer A. Solutions were made fresh daily and stored at 4°C.

The free ^{125}I released from [^{125}I]T_4 was used to measure enzyme activity of stoichiometric T_4 deiodination, as reported by Pazos-Moura et al. (21). Four months after the isotope injection studies, tissue homogenates were thawed at room temperature and centrifuged at 400 *g* for 10 min to remove cell debris. No significant residual ^{125}I from the earlier studies was detectable in the tissue homogenates before the enzyme assays. However, theoretically, had it been present, it would have been <0.0002% of the reaction substrate radioactivity.

The reaction mixture consisted of Buffer A, [^{125}I]T_4 (100,000–150,000 cpm), thyroxine (0.5–20 μmol/l), and tissue homogenate (300–400 μg protein) in a total volume of 500 μl, with a pH of 7.0 (6.9–7.1).

Tubes were incubated at 37°C in a shaking water bath, and the reaction was terminated after 120 min by the addition of 500 μl serum and 1,000 μl 10% TCA. The mixture was centrifuged at 1,000 *g* for 10 min at 4°C, and the radioactivity in the supernatant was determined in the same Gamma Trac 1193 counter (TM Analytic) with a 75% counting efficiency and confirmed by HPLC to be >95% free ^{125}I. Repeated washes recovered >97% free ^{125}I from the reaction. The products in the TCA precipitate were extracted with a mixture of ethyl acetate-butanol (9:1) as previously described (24), and then they were separated and identified using HPLC. Nonradioactive T_3, T_4, and rT_3 were used as controls for identification of the peaks. The iodothyronine reaction products generated under these pH and incubation conditions were 92.3% T_3, 1.5% rT_3, and 5.6% diiodothyronines. Therefore inner ring deiodination (4), with its subsequent extremely rapid conversion of rT_3 to diiodothyronine, should not have accounted for more than ~8.0% of the generated free iodine.

All assays were performed in triplicate along with control tubes containing no homogenate. The nonspecific deiodination determined in these control tubes was minimal and subtracted as background. The enzyme assays were carried out within 14 days of each other with a single animal studied on a given day. Each animal's homogenate was selected randomly after alternation between control and cold treatment groups. An aliquot of stock homogenate was used to study the effect of freezer storage (−70°C). No detectable difference could be found in the activity of the stock homogenate over 1.5 mo of storage, which is in agreement with others who report that the enzyme stored in this manner is stable for up to 1 yr (30). Data were analyzed by the double reciprocal method of Lineweaver and Burk for estimates of the apparent Michaelis-Menten constant (K_m) and maximum enzyme velocity (V_{max}) during the period when the reaction rate was constant. This incubation period used ~10% of the substrate and represents the linear portion of the velocity curve, which is also in agreement with that of others (8, 20). A linear model, using least-squares regression, was fitted to the individual data. This model correlated well with the actual data, indicated by a $P < 0.001$ level of significance for the majority of fits. A single assay for one animal (*B-3*) yields the equation [(velocity)$^{-1}$ in pmol I·min^{-1}·mg protein^{-1}] = 1.030 ± 0.093 × (substrate in μmol/l)$^{-1}$ +0.999 ± 0.085 (pmol I·min^{-1}·mg protein^{-1})$^{-1}$ and is representative of the assay. The mean CV for triplicate assays was 14.5% for V_{max} and 13.5% for K_m determination, and these CVs did not differ between groups. Protein concentration was measured spectrophotometrically by the Bradford method. Tissue 5'D-I activity was identified by 6-*n*-propyl-2-thiouracil (PTU) (Sigma Chemical) inhibition experiments. PTU, at a concentration of 10 mmol/l, inhibited ~90% of the ^{125}I release in this tissue preparation, thus minimizing the possibility of contributions from type II or type III enzyme activity in this assay.

Statistical analysis. Comparisons were made across treatment groups with a nonpaired Student's two-tailed *t* test. Linear regression was performed for correlation coefficients, and all analyses were carried out using microcomputer routines (STATPAK version 4.1, Northwest Analytical, Portland, OR). The data are expressed as means ± SE. $P < 0.05$ is considered significant unless otherwise stated.

RESULTS

Physiological and anatomic measurements. The cold-exposed animals consumed more dietary energy than the controls ($P < 0.007$), although the body weights and growth rates were not significantly different between groups (Table 1). The percent adipose, calculated from the body weight of the living pigs, was similar in both groups. The liver weight in the cold group tended to be greater than in controls but not quite significantly greater ($P = 0.051$). The absolute weight of the thyroid in cold-exposed animals was 86% greater than in control animals ($P < 0.004$). The amounts of protein, lipid, and water in thyroid and liver tissues were not different between control (14, 2.3, and 79% thyroid and 19, 1.1, and 74% liver) and cold-exposed animals (11, 1.8, and 83% thyroid and 18, 0.7, and 73% liver), respectively, when expressed as percentages of wet weight.

Table 1. *Total energy intake, organ and body weights, growth rates, and percent body fat for control and cold-exposed animals*

	Control	Cold
n	5	5
Body weight, kg	72.2 ± 3.2	81.0 ± 3.2
Liver weight, kg	2.6 ± 0.2	3.1 ± 0.1*
Thyroid weight, g	9.56 ± 0.32	17.77 ± 1.99†
Lipid content, %	21.7 ± 0.27	22.3 ± 0.30
Energy intake, g/day	2525 ± 277	3817 ± 216†
Growth rate, kg/day	1.17 ± 0.13	1.02 ± 0.10

Values are means ± SE of 5 boars in each treatment temperature after 25 days of exposure to either 22 or 4°C. Wet weights of liver and thyroid and %body lipid were determined by in vivo ^3H_2O injection, mean energy intake/day at completion of study, and linear regression of growth rate over study period. Significantly different from control: *$P = 0.051$; †$P < 0.007$.

Blood volume and plasma volume were not significantly different between groups, although the total blood volume tended to be greater in the cold-exposed group (Table 2). Hct ($P < 0.04$) and hemoglobin ($P < 0.05$) were increased in the cold-exposed group compared with the control group, suggesting a slight increase in red cell mass. During exposure of the two groups to their respective temperatures, the skin temperature of the ear was reduced by 16.5 ± 2.3°C in the cold-exposed group ($P < 0.0002$), whereas the rectal temperature was not different between groups.

Thyroid hormone values. Serum TT_4 [47.31 ± 2.18 vs. 31.96 ± 3.97 nmol/l (48%)], FT_4 [7.59 ± 0.81 vs. 4.72 ± 0.46 pmol/l (61%)], TT_3 [1.43 ± 0.11 vs. 0.70 ± 0.04

Table 2. *Physiological measurements of temperature, plasma and blood volume, and hematocrit for control and cold-exposed animals*

	Control	Cold
T_{sk}, °C	34.20 ± 0.58	17.75 ± 2.49†
T_{re}, °C	38.40 ± 0.19	39.20 ± 0.20
PV, liters	5.67 ± 0.38	5.82 ± 0.49
BV, liters	7.92 ± 0.52	8.98 ± 0.73
Hct, %	29.40 ± 1.80	34.40 ± 0.67*
Hb, g/dl	9.94 ± 0.66	11.62 ± 0.20*

Values are means ± SE of 5 boars in each treatment temperature after 25 days of exposure. T_{sk}, ear skin temperature; T_{re}, rectal temperature; PV, plasma volume determined with Evans blue dye injection; BV, blood volume; Hct, hematocrit; Hb, hemoglobin. Significantly different from control: *$P < 0.05$; †$P < 0.0002$.

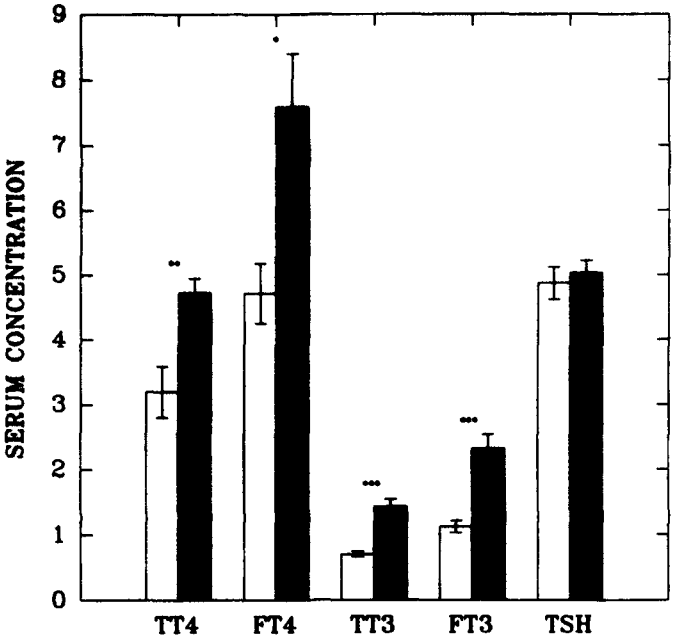

Fig. 1. Values of serum total thyroxine (TT_4) in nmol/dl, free thyroxine (FT_4) in pmol/l, total triiodothyronine (TT_3) in nmol/l, free triiodothyronine (FT_3) in pmol/l, and thyrotropin (TSH) in mU/l for 5 boars (means ± SE) in each treatment temperature after 25 days of exposure to either 22 (open bars) or 4°C (filled bars). T_3, 1.536 × μg = nmol; T_4, 1.287 × μg = nmol. $^*P < 0.02$; $^{**}P < 0.01$; $^{***}P < 0.002$.

nmol/l (103%)], and FT_3 [2.31 ± 0.22 vs. 1.12 ± 0.09 pmol/l (107%)] increased in the cold-exposed group compared with control, whereas mean serum TSH (5.03 ± 0.19 vs. 4.87 ± 0.25 mU/l) was not different between the groups (Fig. 1). Three tissues were studied for their relative iodothyronine contents of T_4 and T_3 in a few animals (n = 1–3 for each assay). The molar ratio of T_4 to T_3 (T_4/T_3) in the tissues from control animals was, for liver, 15.0:1 (1); thyroid, 108.2:1 (1); and skeletal muscle, 0.059:1 (2). In cold-exposed animals the ratio was, for liver, 7.30:1 (1); thyroid, 37.9:1 (3); and skeletal muscle, 0.053:1 (1). The cold-exposed animals showed a reduction in T_4/T_3 by 51.3% for liver, 65.0% for thyroid, and 10.2% for skeletal muscle. When the cold treatment changes for liver and thyroid were pooled and compared with those of muscle, this difference was 48.0 ± 6.9% greater for liver and thyroid ($P < 0.02$).

Serum kinetic parameters. The mean serum values for the two groups of animals are presented in Fig. 2, together with a two-exponential fit to the mean data as used with the NC analysis of the data for individual pigs.

The compartmental parameters resulting from the initial "limiting" fits of the three-compartmental model (Fig. 3) to the serum data are presented in the first two segments of Table 3. These values can be considered outer bounds for these parameters. The third segment of Table 3 shows the compartmental parameters for the model solutions in which we assume a midpoint value for $k_{2,1}$, with separate solutions of the exit parameters from both peripheral compartments. Although there appears to be a trend toward slower exchange rates for both peripheral compartments in the cold-exposed animals, this is not statistically significant. However, the increased rate of exit from the cold-exposed animals' slow-exchange compartments (noted in the third solution set) is significant ($P < 0.05$).

Table 4 presents the steady-state kinetic parameters calculated from the third (combined) set of these model parameters for comparison with the results of the NC model steady-state parameters derived from the eigen

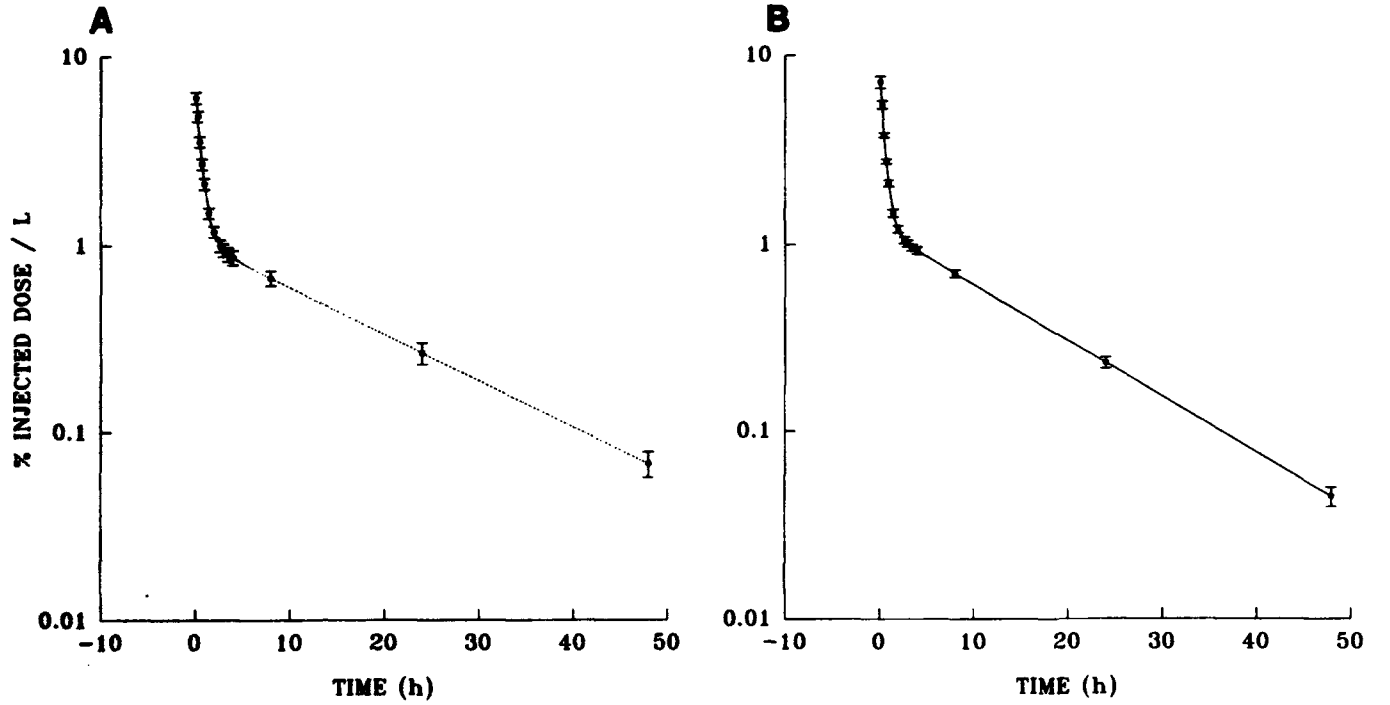

Fig. 2. Fitted serum [^{125}I]-T_3 disappearance curves (means ± SE) expressed as percentage of injected dose/l for 5 control animals (· · · ·, *A*) and 5 cold-exposed animals (——, *B*).

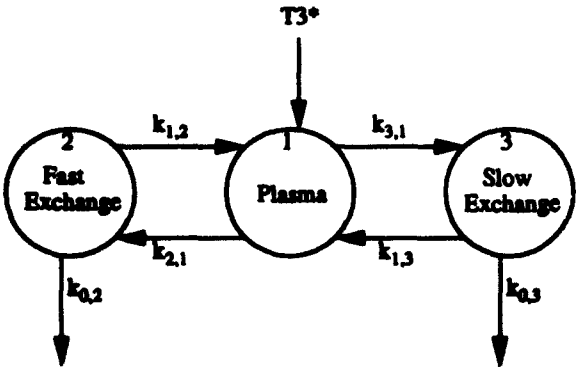

Fig. 3. Three-compartment mammillary model of [^{125}I]T_3 (T_3*) with corresponding rate constants listed in Table 3.

values of the two-exponential fits. The steady-state T_3 content and clearance values, calculated from the steady-state kinetic parameters and the observed serum T_3 concentrations, are also presented. The kinetic steady-state parameters — the plasma-equivalent volumes of distribution (V), plasma clearance rates (PCR), as well as the fractional clearance rates (FCR) and mean residence times (MRT) — show little, if any, change with cold exposure. There is a marginally significant ($P < 0.05$) decrease in V in the slow-exchange tissues, which is balanced by an increase in FCR ($P < 0.05$) from these same tissues. The product of V and FRC is the PCR, which remains unchanged with cold exposure. The FCR and MRT data are given later in the text.

On the other hand, as might be expected, when V and PCR are multiplied by the serum T_3 concentrations to calculate the Qs and PRs, the increased circulating T_3 levels in the cold-exposed animals are reflected in marked increases in Q and PR.

Table 3. *Parameter values resulting from solution of three-compartment model*

	Control	Cold
	All exit from compartment 2	
$k_{2,1}$	12.9 ± 2.3	7.6 ± 1.4
$k_{1,2}$	6.0 ± 1.2	3.1 ± 0.7
$k_{3,1}$	2.2 ± 0.2	1.7 ± 0.1
$k_{1,3}$	0.24 ± 0.03	0.23 ± 0.02
$k_{0,2}$	0.43 ± 0.02	0.44 ± 0.06
	All exit from compartment 3	
$k_{2,1}$	11.7 ± 2.2	6.8 ± 1.3
$k_{1,2}$	6.1 ± 1.0	3.6 ± 0.7
$k_{3,1}$	3.1 ± 0.2	2.6 ± 0.1
$k_{1,3}$	0.17 ± 0.03	0.15 ± 0.02
$k_{0,3}$	0.063 ± 0.003	0.074 ± 0.008
	Exit from both compartments 2 and 3	
$k_{2,1}$	12.3 ± 2.2	7.2 ± 1.3
$k_{1,2}$	6.2 ± 1.1	3.4 ± 0.7
$k_{3,1}$	2.7 ± 0.2	2.2 ± 0.1
$k_{1,3}$	0.20 ± 0.03	0.18 ± 0.02
$k_{0,2}$	0.21 ± 0.01	0.21 ± 0.02
$k_{0,3}$	0.038 ± 0.002	0.052 ± 0.006*

See model (Fig. 3). Three solutions (h^{-1}) are shown, representing 2 extreme solutions and consolidated model resulting when $k_{2,1}$ was fixed at its midpoint for the individual pig for 2 extreme solutions and both $k_{0,2}$ and $k_{0,3}$ were allowed to fit. *$P < 0.05$ vs. control.

Table 4. *In vivo serum T_3 kinetic parameters of control and cold-exposed animals calculated by both multicompartmental and noncompartmental methods*

	Control	Cold Exposed
V, l/kg		
Compartment 1 (plasma)	0.079 ± 0.005	0.073 ± 0.008
Compartment 2 (fast)	0.16 ± 0.02	0.14 ± 0.01
Compartment 3 (slow)	0.93 ± 0.09	0.69 ± 0.02*
Total compartmental	1.17 ± 0.11	0.91 ± 0.02*
Noncompartmental	0.95 ± 0.07	0.72 ± 0.04*
Q, nmol/kg		
Compartment 1 (plasma)	0.055 ± 0.001	0.113 ± 0.010‡
Compartment 2 (fast)	0.11 ± 0.02	0.22 ± 0.03†
Compartment 3 (slow)	0.65 ± 0.07	1.10 ± 0.10†
Total compartmental	0.81 ± 0.07	1.43 ± 0.4†
Noncompartmental	0.66 ± 0.05	1.01 ± 0.06†
PCR, $l \cdot kg^{-1} \cdot day^{-1}$		
Compartment 2 (fast)	0.79 ± 0.09	0.68 ± 0.06
Compartment 3 (slow)	0.84 ± 0.09	0.86 ± 0.10
Compartmental whole body	1.63 ± 0.17	1.55 ± 0.12
Noncompartmental	1.56 ± 0.15	1.43 ± 0.10
PAR, $nmol \cdot kg^{-1} \cdot day^{-1}$		
Compartment 2 (fast)	0.56 ± 0.07	1.07 ± 0.11†
Compartment 3 (slow)	0.58 ± 0.05	1.33 ± 0.13‡
Compartmental whole body	1.14 ± 0.11	2.40 ± 0.16‡
Noncompartmental	1.10 ± 0.11	2.01 ± 0.11‡

Steady-state parameters derived from 3rd set of 3 compartment model solutions presented in Table 3 and observed steady-state serum triiodothyronine (T_3) content, presented in comparison with results of an independent noncompartmental analysis. Plasma-equivalent volume of distribution (V), T_3 content (Q), plasma-equivalent clearance rate (PCR), and T_3 plasma appearance rate (PAR) values represent minimum bounds (see text) for those values. All results are presented as means ± SE of 5 animals. Significantly different from control: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

When the total body results of the three-compartment and NC solutions are compared, the Vs and PCRs and their T_3 counterparts, the Qs and PRs, are greater with the three-compartment models, as expected. Also, as noted above, even the MC solution gives only lower bounds for V, Q, PCR, and PR, the upper bounds being 26 and 36% higher in the control and cold-exposed pigs, respectively.

FCR measured in percent per day, which we would expect to be equivalent in the two methods, is smaller in the MC solutions (cold vs. control): FCR_{fast} 504 ± 52 vs. 512 ± 28; FCR_{slow} 124 ± 14 vs. 91 ± 5 ($P < 0.05$); $FCR_{whole\ body}$ 171 ± 12 vs. 140 ± 6 ($P < 0.05$); FCR_{NC} 198 ± 8 vs. 166 ± 10 ($P < 0.05$). There is a corresponding lengthening of MRT with the MC over the NC solutions measured in hours: MRT_{fast} 5.0 ± 0.6 vs. 4.7 ± 0.2; MRT_{slow} 20.3 ± 1.8 vs. 26.8 ± 1.3 ($P < 0.05$); $MRT_{whole\ body}$ 14.4 ± 0.9 vs. 17.3 ± 0.8 ($P < 0.05$); MRT_{NC} 12.2 ± 0.5 vs. 14.7 ± 1.0 ($P < 0.05$). These differences are probably due to the choice of a two-exponential fit of the data for the NC solutions, which tends to underemphasize the late flattening of the decay slope.

The calculated experimental changes were in the same direction and of magnitude comparable to the different model solution methods. We believe, therefore, that these discrepancies are not important in interpretation of the experimental results and, unless specified, we will refer to the NC solutions.

5'D-I activity. The Lineweaver-Burk plots support a major effect on V_{max} shown by the change in the *y*-intercept and slope and a minor effect on the K_m represented by the *x*-intercept (Fig. 4) that are upheld in the analysis of individual animal parameters. In the cold-exposed group, V_{max} of 5'D-I was increased to 2.50 ± 0.34 pmol $I \cdot min^{-1} \cdot mg\ protein^{-1}$ compared with the mean control group value of 1.21 ± 0.25 pmol $I \cdot min^{-1} \cdot mg\ protein^{-1}$; $P < 0.02$. There was no significant difference in K_m between cold (2.22 ± 0.33 μmol/l) and control (1.88 ± 0.33 μmol/l) groups. The ratio expression of V_{max}/K_m (30) was different between groups and increased ~70% in the cold-exposed swine ($P < 0.01$) compared with control [1.15 ± 0.06 vs. 0.68 ± 0.12 pmol $I \cdot min^{-1} \cdot mg\ protein^{-1} \cdot (\mu mol/l)^{-1}$].

Correlations. A significant positive correlation with a linear regression ($n = 10$) was determined between PAR and thyroid size ($r = 0.818$, $P < 0.002$; Fig. 5), between PAR and serum TT_4 ($r = 0.761$, $P < 0.005$), and between PAR and hepatic 5'D-I activity ($r = 0.564$, $P < 0.044$). Serum TT_4 was not significantly correlated with 5'D-I activity.

DISCUSSION

Twenty-five days of continuous cold exposure doubles serum TT_3, FT_3, thyroid weight, T_3 PR, and hepatic 5'D-I V_{max}, with smaller increases in TT_4 and FT_4, whereas serum TSH remains unchanged. Energy intake increased ~50% in the cold-exposed animals to maintain growth rates similar to those of controls. These findings establish that the physiological response of cold-exposed adult male swine involves an increased T_3 production, with hepatic 5'D-I probably having a significant role in this adaptation.

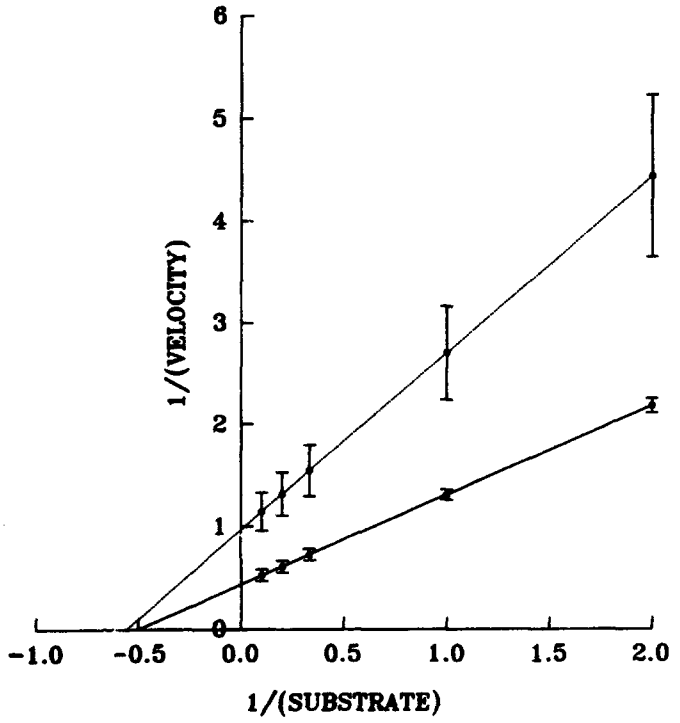

Fig. 4. Lineweaver-Burk plots of 5'-deiodinase type I (5'D-I) activity in hepatic homogenates from 5 control (○) and 5 cold-exposed (●) animals. Each data point represents fitted velocities (means ± SE) from triplicate assay for each of 5 animals and pooled for each group, with summary regression lines presented for comparison.

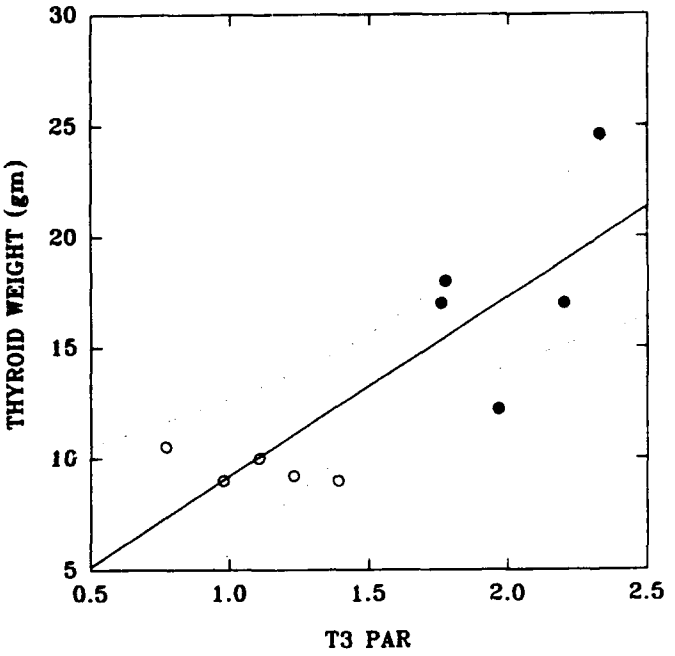

Fig. 5. Linear least-squares regression (solid line) of individual animal thyroid gland weight and in vivo plasma appearance rate (PAR, $nmol \cdot d^{-1} \cdot kg^{-1}$). Each point represents 1 of 10 individual boars in either control (22°C, ○) or cold (4°C, ●) condition after 25 days. The 95% confidence regions of regression are represented by broken lines ($r = 0.818$, $P < 0.002$).

This swine model (6, 11, 16) mimics some human responses. Men exposed to cold (~4–11°C) increase oxygen utilization. If they are subjected to these temperatures for 12–14 days while partially clothed, thyroidal iodine clearance and serum $[^{125}I]T_4$ removal increase (15). Furthermore, as recently reported with this type of cold exposure, human T_3 production and clearance increase independent of TSH, even though humans have little contribution from BAT (1, 23). Our major objective was to study the influence of cold on T_3 kinetics with animals in energy balance. Cold-exposed swine maintain growth rates by increasing their energy intake (12, 13, 18); thus feed consumption cannot be isolated from the other effects of cold in animals fed ad libitum as done in our study. This study, therefore, cannot separate the causal relationship between the two variables of cold exposure and increased energy intake on T_3 generation. The increased energy intake was necessary to maintain nutritional balance as defined by the similar amounts of body fat and rates of growth of the two groups. Calorie restriction, on the other hand, decreases hepatic T_3 production (27) and in vivo T_4 degradation (9) in swine. Consequently, equalizing the energy intake between treatments to that of the control group would result in possibly confounding conditions. Further studies that limit energy intake during cold exposure will need to be carried out to expand our understanding of this relationship.

The increased serum concentrations of TT_3, FT_3, and TT_4 with cold exposure are similar to those reported in

younger swine living for 3 wk in temperatures between 10 and 12°C (12, 13). Macardi has shown in thyroidectomized swine that increased serum thyroid hormone values are critical for sustaining the increased energy intake found during cold exposure (18). Taken together, these earlier observations and our findings support the hypothesis that increases in thyroid hormone production are adaptive physiological responses to cold exposure associated with increased energy intake and a normal growth rate.

Contrary to Herpin et al. (12), we do not find a significant difference in the TSH values between the groups. Animal maturity, circadian fluctuation, or energy balance at the time of death may account for this discrepancy between studies in serum TSH concentrations. Alternatively, homeostatic feedback should result in decreased serum TSH when circulating free T_4 is markedly elevated, suggesting the possibility of decreased pituitary sensitivity in both studies. Conversely, thyroidal enlargement may be mediated by small changes in TSH, antibodies to the TSH receptor, or the sensitivity of the gland to growth factors. Unfortunately, we have no histological assessment of these glands to help clarify the mechanism of enlargement. The sensitivity of this porcine TSH assay may not be sufficient to detect small differences between groups, and thus subtle changes may be missed.

The elimination rate of [^{125}I]T_4 doubles if energy intake is allowed to increase in 8- to 12-wk-old swine exposed to cold (8°C) for 4 days (6). However, when energy intake is restricted to that of the control period, the T_4 removal rate with cold is also similar to that of the control period (6, 16). Because increased energy intake is required by swine during exposure to cold, then these data reported by both Evans and Ingram (6) and Ingram and Kaciuba-Uscilko (16) are in general agreement with human studies by Suda et al. (28) and with swine studies by Griggio and Ingram (9). These findings may be explained by a degree of environmental undernutrition interacting with cold exposure to result in a relatively decreased T_4 plasma clearance rate, a mechanism possibly mediated by decreased 5'D-I activity. Gastrointestinal loss of conjugated T_4 and T_3 through bile secretion is usually small but may increase in parallel with energy intake and therefore represent another possible mechanism to explain these changes.

The serum [^{125}I]T_3 kinetic parameters in our study are difficult to compare with other published porcine data (5, 9). These earlier studies used less mature animals and terminated the experiment 3 h after injection, reporting only a single rate constant. This type of analysis is limited by a single distribution rate constant model for T_3 kinetics. The more complex kinetics of T_3, with slow or late plasma disappearance, is not accounted for in earlier reports (Fig. 2) (5, 9). The values for control swine that we provide using NC techniques are similar to canine and feline values calculated over a period to include both rate constants by use of MC techniques (10).

The major increase in T_3 Q in the face of little or no change in V is supported by the in vitro assay of these tissues after administration of a bolus [^{125}I]T_3 dose 3 h before death, as previously reported (22). The serum-to-tissue ratio of the tracer was similar for most tissues, resulting in increases in Q_{tissue}. An exception was subcutaneous hip fat, which showed an increased uptake even though the extraction coefficient was constant, suggesting a subtle tissue-dependent distribution effect not detectable with our NC analysis of the kinetic data. The three-compartmental analysis using fast, slow, and plasma compartments suggests that the increase in the tissue FCR of T_3 noted in the NC solution is due to the slowly exchanging tissues such as fat. The increase of in vivo plasma T_3 appearance rate that we describe may be associated with either increasing thyroidal T_3 production or with an augmented peripheral production of T_3 by 5'D-I. Factors such as immunoglobulins (19) or sympathetic nervous system stimulation that may shift thyroid gland or peripheral 5'D-I hormonogenesis to favor T_3 production have not been eliminated by this study.

The relative tissue content of T_4 and T_3 appears to change with cold exposure in two of the three tissues studied (liver and thyroid). The tissue uptake of T_4 is not likely decreased by cold air treatment (6), and T_3 uptake is not increased from these tissues using our compartmental analysis or in vitro techniques (22). The apparent decline (~50%) in T_4/T_3 implies either increased tissue production of T_3, increased degradation of T_4, or both. The two tissues that decrease this ratio are liver and thyroid, both of which have very effective 5'D-I activity. Swine skeletal muscle, on the other hand, has only ~3% the activity per gram of protein as swine liver (26) and shows little change during the same period of cold exposure. These preliminary results suggest to us that the thyroid with its 5'D-I activity may have a significant role in contributing to the increased in vivo T_3 production rate found in the present study.

We are not able to distinguish the sequence for increasing T_4 (6) and T_3 production in swine fed ad libitum. Two possibilities seem likely: *1*) the increased serum T_4 production stimulates an increase in 5'D-I activity and production of T_3, or *2*) increased 5'D-I activity stimulated by a circulating substance increases T_3 production in tissues with known 5'D-I activity such as liver, kidney, and thyroid, whereas the thyroid alone, responding as if to TSH, also produces T_4.

The activity of 5'D-I is PTU sensitive, whereas type II iodothyronine 5'-deiodinase activity is relatively unaffected by PTU (8). PTU inhibition of the enzyme that we assayed characterizes it predominately as type I, which is commonly found to be decreased in fasted swine (27). Type I enzyme activity in rodents is increased with hyperthyroidism, decreased with hypothyroidism, and variable with cold exposure (20, 21). A cold stimulus and fixed T_4 replacement in hypothyroid animals increases 5'D-I even though serum T_4 values may be very low, suggesting that the regulation with cold exposure is not dependent on increased circulating T_4 concentrations (20). In contrast, however, Pazos-Moura et al. (21) found no change in hepatic 5'D-I activity compared with

large changes in BAT 5'D-II activity of iodine-deficient rats replaced with iodine and exposed to cold.

Our values for V_{max}/K_m [0.68 ± 0.12 pmol I·min^{-1}·mg protein^{-1}·(μmol/l)$^{-1}$ in the control group] are in general agreement with values determined in swine up to age 42 days (26), where thyroxine was also used as a substrate for this enzyme. Differences in V_{max} between studies may depend on the age of the animals (26) and the state of nutrition at death because only moderate energy restriction results in "an approximately threefold decrease in the net T_3 produced by the liver homogenates" (27). These earlier studies also differ from our own in that the reaction product T_3 was measured by RIA (3, 26, 27), possibly contributing to the substantial within-group variability of adult animals (26).

Hepatic and renal contributions to daily T_3 in vivo production have been reported to be between 50 and 80% (14). Because the liver and kidneys together are thought to account for the majority of the fast-exchanging tissues assessed in the three-compartment model, this model form gives additional information about the distribution of T_3 production and disposal. PR in our control animals was approximately the same in the fast and the slowly exchanging compartments, but the fraction attributable to the slowly exchanging tissues increased slightly (from 51 to 55%) in the cold-exposed animals.

Calculations from absolute in vitro enzyme activity involving many estimated variables predict only 16% of total daily T_3 production (30). This inability to predict accurately the in vivo T_3 production may be attributed to in vitro studies that do not use natural cofactors such as reduced glutathione. Another reason for these discrepancies might be that the concentration of the substrate near the enzyme is increased with an active pump that would be present in whole organ preparations and not in these assays. Thus, to help normalize these variables, we have expressed this prediction as a percent change from control. Assuming that liver has similar T_4-binding coefficients, and knowing that the percent protein and total weight of the liver are similar in the two groups, we can estimate the hepatic T_3 production as $FT_4 \times (V_{max}/K_m)$ (30) and then express a percent change between the two groups. The 70% increase in V_{max}/K_m in the cold group, combined with the increased FT_4, would represent a 174% increase in hepatic 5'D-I T_3 production over the control group. With the assumption that 50% of the in vivo T_3 PR is from a 5'D-I-mediated conversion of T_4 in liver, kidney, and possibly thyroid, as well as slow-compartment contributions of skeletal muscle and fat, the control group would contribute ~0.55 (NC) and ~0.63 (MC) nmol·day^{-1}·kg^{-1} from this enzymatic mechanism. Increasing the activity of 5'D-I equally in both the fast and slow compartments after cold exposure in a fashion similar to that found in the liver could then contribute ~1.51 (NC) and ~1.73 (MC) nmol·day^{-1}·kg^{-1}. Although this contribution is speculation, the change would more than account for the observed differences between the two treatment groups of 0.91 (NC) and 1.26 (MC) nmol·day^{-1}·kg^{-1}.

In conclusion, we report that, after 25 days of cold exposure, T_3 production was increased along with thyroidal size and the deiodination of thyroxine by hepatic 5'D-I. During cold exposure, increased serum T_4 concentrations and thyroid weights were not associated with changes in serum TSH. Further study is required to completely understand the time course of trophic events and the target tissue relevance of these findings.

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Experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS Publication (NIH) 86–23 (1985).

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